1.0 INTRODUCTION

Vical Inc. recently completed enrollment in a Phase I/II, clinical study entitled *Phase I Trial Of Interleukin-2 DNA/DMRIE/DOPE Lipid* Complex *As An Immunotherapeutic Agent In Solid Malignant Tumors Or Lymphomas By Direct Gene Transfer.* The study was conducted at the Arizona Cancer Center and Scott and White Clinic in which safety was evaluated in a dose escalation regimen in 23 cancer patients with metastatic disease. Analysis of the data collected from the study indicates that the product, Leuvectin, is safe and non-toxic administered intratumorally. Five groups of patients were studied. Each patient received a single, intratumoral injection of Leuvectin each week for 6 weeks. Each patient group received a single dose level, either 10, 30, 100 or 300 μg of Leuvectin. A maximum tolerated dose was not identified. Further details of the trial are contained in section 2.3.

Vical now proposes an additional Phase I dose escalation study to evaluate 3 doses of Leuvectin with each dose administered intralesionally in two series of injections in patients with metastatic melanoma, renal cell carcinoma and sarcoma. In this study, the doses will include 300, 750 and 1500 μg of Leuvectin per injection. The objectives are to further evaluate safety, determine whether the study agent evokes an immune response and evaluate any effect of the agent or the induced immune response on disease status.

2.0 BACKGROUND AND RATIONALE

Immunotherapy has shown promise as an approach to the treatment of malignancy. The goal of immunotherapy is to stimulate the immune system to recognize and kill cancer cells. This may be achieved by modifying either the tumor cells or the host response causing various lymphocyte populations, particularly cytotoxic T lymphocytes (CTLs), to respond specifically to tumor cell antigens- Cancers such as melanoma and renal cell carcinoma are sometimes responsive to immunotherapy because the immune system can be induced to recognize tumor-associated and tumor-specific antigens in these cells.

In some instances, the immune system appears to contribute to the surveillance and destruction of neoplastic cells by mobilization of either cellular or humoral immune effectors. Cellular mediators of antitumor activity include MHC-restricted cytotoxic T cells (CTLs), natural killer (NK) cells (1, 2) and lymphokine-activated killer (LAK) cells (3). Cytotoxic T cells which infiltrate tumors have been isolated and characterized (4). These tumor infiltrating lymphocytes (TIL) selectively lyse cells of the tumors from which they have been derived (5, 6). Macrophages can also kill neoplastic cells through antibody-dependent mechanisms (7, 8), or by activation induced by substances such as Bacillus Calmette-Guerin (BCG) (9).

Cytokines also participate in the antitumor response by direct action on cell growth or by activating cellular immunity. The cytostatic effects of tumor necrosis factor-α (TNF-α), interferon-α (IFN-α), interferon-γ (IFN-γ) and lymphotoxin can result in neoplastic cell death (10, 11). Interferon-γ markedly increases class I and II MHC cell surface expression (12, 13) and synergizes with TNF-α in producing this effect (14). Colony stimulating factors such as G-CSF and GM-CSF activate neutrophils and macrophages to lyse tumor cells directly (15), and interleukin-2 (IL-2) activates Leu-19+ NK cells to generate lymphokine activated killer cells (LAK) capable of lysing autologous, syngeneic or allogeneic tumor cells but not normal cells (3, 16, 17). The LAK cells lyse tumor cells without preimmunization or MHC restriction (18). Interleukin-4 (IL-4) also generates LAK cells and acts synergistically with IL-2 in the generation of tumor-specific killer cells (19).

Systemic administration of IL-2 alone, or IL2 with LAK cells has been shown to upregulate the immune system resulting in tumor regression (20). Recently, several studies have examined the tumor suppressive effect of lymphokine production by genetically altered tumor cells. The introduction of murine melanoma B16, murine fibrosarcoma CMSS and murine B cell lymphoma 38C13 tumor cells transduced with an IL2 expression vector into syngeneic mice stimulated an MHC class I restricted cytolytic T lymphocyte (CTL) response which protected against subsequent rechallenge with the parental tumor cell line (21). Immunological memory was confirmed by implanting lethal doses of tumor cells several months after the initial tumor regression/cure. The implanted cells were rejected and specific CTLs which were capable of killing tumor cells *in vitro* were found in the spleens of the mice.

2.1 Direct Gene Transfer Method for Modulation of the Immune System: Preclinical Pharmacology and Toxicology Studies

Several gene therapy approaches have involved genetic modifications of tumor cells *in vitro* followed by reintroduction *in vivo* (22). Scientists at Vical Inc have developed a direct gene transfer method to transfect tumor cells with genes encoding for immunomodulating proteins. The Vical approach introduces the recombinant gene encoding the IL-2 protein directly into malignant tumor cells *in* . *vivo* which eliminates the need to establish cell lines from each patient and minimizes delays in the time to treatment. Additionally, no viral vectors are contained in the formulation. The product, Leuvectin, is composed of the plasmid DNA coding for IL-2 (VCL-1102) formulated in an injection vehicle with DMRIE/DOPE, a proprietary cationic lipid mixture (cytofectin). When introduced into the target tumor, the lipid facilitates transfection of the tumor cells. The IL-2 gene product is expressed and secreted at the tumor site.

In developing Leuvectin, Vical conducted pharmacology and toxicology studies to 1) demonstrate that the plasmid/lipid complex was biologically active *in vivo* and produced IL-2 protein in tumors, 2) demonstrate that VCL-1102 reduced tumor burden in a mouse tumor model, and 3) explore the effect of injecting the plasmid directly into normal mouse liver. Results of the studies showed that 1) the IL-2 plasmid/lipid formulation produces a high level of IL-2 protein expression in injected tumors, 2) the direct intratumoral injection of a plasmid DNA expression vector encoding the human IL-2 gene into subcutaneous B16 melanoma or renal cell carcinoma tumors in mice significantly slowed tumor growth and reduced the incidence of palpable tumors, and 3) intrahepatic administration of Leuvectin in mice was well tolerated and there were no adverse effects associated with the drug adverse effects that occurred were attributed to the injection procedure itself and not considered to be of biological significance because no dose related response was observed Details of the pharmacology and toxicology studies are contained in the Investigator's Brochure.